

GENOMIC APPROACHES AND GERMPLASM DIVERSITY FOR CHICKPEA IMPROVEMENT

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Abstract

The paper aimed to present different genomic approaches and the role of *Cicer arietinum* diversity in breeding. The chickpea is one of the promising pulse crops in Europe. Recent European strategies focused on imperative need of safety and secure food and clean environment highlighted the importance of new and performant pulses genetic resources available for farmers. Despite the nutritional, agronomic and environmental benefits of chickpea, this crop remains insufficient explored. The low level of European plant protein self-sufficiency is due to: the lack of breeding resources and knowledge gaps (low agronomic expertise, insufficient cooperation between stakeholders, non-competitive management of PGR), poor adaptation of protein plant cultivars in Europe. The study presents: (1) modern breeding approaches based on genomic approaches, (2) the diversity of chickpea by screening of the vast volume of accessions available in gene banks, (3) the role of inclusion of wild genetic material in current breeding programs, thanks to their feature to imprint tolerance/ resistance to different abiotic and biotic stressors.

Key words: sustainable agriculture, wild germplasm, crop development, genetic variation.

INTRODUCTION

The *Fabaceae* family, also known as *Leguminosae*, stands as the third-largest family of flowering plants. Following cereals, legumes emerge as the most agriculturally significant crop family (Graham & Vance, 2003), serving diverse purposes, including aquaculture feed, animal forage and human food. Leveraging their symbiotic nitrogen-fixing traits, legumes play a vital role in both natural ecosystems and sustainable agriculture (Afzal et al., 2020). They contribute to crop rotations and enhance soil fertility, especially in arid regions and areas with low nitrogen levels (Zahran, 1999; Appelbaum, 2018). Climate change affects agricultural systems, ranging from changes in water availability, flowering phenology, soil fertility, and erosion, to an escalation in the spread of pathogens and increased susceptibility of hosts (Rosenzweig, 2015; Jimenez-Lopez et al., 2020). It also leads to more nuanced alterations in plant distribution, biodiversity, and interactions between plants and pollinators (Bishop et al., 2016). However, when it comes to crop development and breeding, a more comprehensive approach involves identifying climate-related shifts in the

biodiversity of crop wild relatives (CWR) alongside assessing farming suitability. To achieve faster greater genetic gains, it is necessary to improve the precision and efficiency of segregation in generations. Screening under controlled environmental conditions or at hot spot locations can improve precision in selection for resistance/tolerance to stresses (Gaur et al., 2012). Chickpea (*Cicer arietinum*) stands as one of the most ancient pulse crops, with cultivation dating back to before 9500 BC. Widely grown in over 50 countries worldwide (Upadhyaya et al., 2011), chickpea is traditionally cultivated as a low-input crop, in conditions of diminishing soil moisture and minimal management. Despite its significant morphological diversity, there is limited genetic variation in chickpea (Udupa et al., 1993), likely due to its monophyletic divergence from its wild progenitor *C. reticulatum* (Abbo et al., 2003). Chickpea cultivation is predominantly found in arid and semi-arid regions spanning the Mediterranean basin, Central Asia, East Africa, Australia, Europe and North and South America (Bar-El et al., 2017). In these areas, chickpea faces significant susceptibility to abiotic stresses like drought and heat at different growth stages

throughout the productive season (Croser et al., 2003; Maphosa et al., 2020). Therefore, there is a critical need for breeders to identify and/or develop highly productive chickpea genotypes through a combination of breeding methods. The new chickpea cultivars should exhibit resilience to climate change, possess genetic diversity, demonstrate efficiency, and showcase adaptability across various environments. This is crucial for ensuring food security in the foreseeable future (Mba, 2013). Given the limited genetic diversity in the cultivated species, pre-breeding becomes pivotal in the genetic enhancement of chickpea. Introducing desirable genes/alleles from wild germplasm into cultivated chickpea can enhance tolerance to abiotic stress and boost yields, thereby contributing to sustained food security in the years ahead (Singh et al., 2021). The selection of novel cultivars should hinge on a comprehensive phenotypic and genetic characterization of materials designated for use as parental resources in breeding programs (Arriagada et al., 2022). This selection process can leverage advanced high-throughput phenotyping techniques (Mir et al., 2019), including the assessment of plant canopy temperature and root system architecture in experiments simulating drought stress and heat (Brunel-Saldias et al., 2020). Complete genetic characterization of parental material ought to be a fundamental cornerstone in the future.

The conservation and utilization of varied collections of plant genetic resources form the foundation of plant breeding programs. This genetic diversity serves as the raw material for the crop breeding industry, where the process of selection operates to develop superior genotypes (Saeed et al., 2011).

MATERIALS AND METHODS

This work is the result of searching and documentation of a significant volume of literature related to the subject of chickpea breeding. 82 open access sources were selected, from SCOPUS and the Google Academic database based on their focus on chickpea diversity and genomic approaches for objectives like improvements in chickpea nutritional quality, yield, and tolerance to a diverse source of biotic and abiotic stresses,

role, and the impact of *ex situ*, *in situ* and *on farm* conservation.

RESULTS AND DISCUSSIONS

Through both traditional and modern plant breeding techniques, it is possible to enhance the genetic diversity of plant species including pulses, cereals, and crucial food crops (Al-Khayri et al., 2019). The existing genetic variability within germplasm, particularly in wild species, can be harnessed to expand the genetic foundation of crop varieties and introduce beneficial traits, such as resistance to pests and diseases. This work presents aspects related modern breeding approaches, the diversity of chickpea reflected by the volume of accessions available in gene banks and the role of inclusion of wild genetic material in current breeding programs. The purpose of these topics is to present aspects to be considered for a successful breeding program.

Modern breeding approaches based on genomic approaches

Obstacles to interspecific hybridization have constrained the exploitation of various wild species, necessitating concentrated endeavours to obtain genes from these species (Dixit et al., 2022). The primary limitation of pulse yields arises from substantial genotype \times environment ($G \times E$) interactions influencing the expression of crucial quantitative traits leading to slow progress in genetic improvement and yield stability of pulses, besides significant damage caused by susceptibility of pulses to biotic and abiotic stress (Kumar and Ali, 2006). These challenges demand immediate attention, prompting a need for a fundamental shift in breeding strategies to reinforce traditional crop improvement programs. A viable approach involves integrating genomics tools into conventional breeding programs, incorporating molecular marker technology for the selection of desirable genotypes or the cultivation of transgenic crops (Kumar et al., 2011). In the past, the breeding of pulses, cereals, and essential food crops, involved leveraging existing genetic diversity through traditional methods like hybridization, mass selection and pedigree selection. However, these approaches are now insufficient to make substantial contributions to

address the escalating global demand for food. The genetic variability in food crops, especially grain legumes, has been depleted, underscoring the need for innovative breeding tools to create new genetic variability in yield traits (Al-Khayri et al., 2019). It is necessary to establish and implement high-throughput precision phenotyping protocols for screening germplasm and breeding materials, particularly targeting traits related to stress tolerance and nutritional quality. Over the past decade, significant progress in chickpea genomic resources has enabled the initiation of genomics-assisted breeding for chickpea improvement. Numerous molecular markers linked to valuable traits have been identified, and some have undergone validation for use in breeding programs (Al-Khayri et al., 2019; Solanki et al., 2022). It is imperative to intensify endeavours to enhance the pool of validated/diagnostic markers, ensuring the integration of genomics-assisted breeding as a comprehensive approach within chickpea breeding initiatives. The adoption of marker-assisted selection holds the potential to expedite the breeding process and streamline the combination of diverse useful traits (Dixit et al., 2022). The process of selecting traits based on phenotype is complex, requiring intricate screening of elite genotypes and proving challenging to execute through conventional methods (Torres, 2009). This underscores the imperative for adopting advanced plant breeding approaches (Al-Khayri et al., 2019).

Transgenic Approach

Transgenic crops become particularly essential for traits that are challenging to genetically enhance using conventional methods, primarily due to the absence of satisfactory sources of desirable gene(s) within crossable gene pools (Kumar et al., 2011; Rasool et al., 2015). The fusion of recombinant DNA technology and plant tissue culture has provided opportunities to create inventive strategies for addressing biotic stress, particularly in the context of pests. These technologies have significantly mitigated losses caused by insects. Biotechnological progress has created a multitude of unique possibilities, including techniques for plant transformation, modifications in gene expression, the discovery of novel and potent molecules and their functions, and the

establishment of transgenic varieties resistant to insect infestation (Al-Khayri et al., 2019). Creating transgenic chickpea lines with resistance to *Helicoverpa armigera* is considered as one of the best strategies to mitigate yield loss. Instances of transgenic events conferring resistance to pod borers have also been observed in chickpea. Incorporating DNA sequences that encode methionine-rich seed proteins through gene transfer technologies presents an appealing avenue for enhancing the protein quality of grain legumes, offering a compelling alternative to traditional methods of pulse breeding (Arya et al., 2022).

Genetic Mapping

The repercussions of climate change and global warming underscore the necessity for researchers to investigate the influence of drought stress on crop growth and productivity. Consequently, it has become essential to cultivate varieties capable of reaching their optimum potential in environments featured by drought stress or dependence on rainfed environments. Given the complexities involved in breeding drought-tolerant cultivars, the discerning identification of quantitative trait loci (QTLs) associated with component traits of drought tolerance can be a useful strategy in the context of chickpea breeding (Kushwah, et al., 2022). Root characteristics, including traits like root length density, volume, depth, and mass, play a crucial role in chickpea's ability to adapt to drought and heat (Kashiwagi et al., 2015). Various quantitative trait loci (QTLs) governing these root traits have been identified (Gaur et al., 2008). Phenotyping root traits accurately poses challenges due to their subterranean growth, making difficult full recovery from soil. Common techniques for characterizing chickpea root traits involve polyvinyl chloride cylinder (PVC) growth systems (Varshney et al., 2013a), soil cores, semi-hydroponic systems (Chen et al., 2017), and shovelomics (Burrige et al., 2016). While effective, these methods are time-consuming and labour-intensive. Advanced image-based root phenotyping methods, such as X-ray computer tomography, magnetic resonance imaging, positron emission tomography, and GROWSCREEN-Rhizo, hold promise for enhancing chickpea germplasm against drought and heat stresses. These methods allow

simultaneous phenotyping of both shoot and root, offering a comprehensive approach (Tracy et al., 2020). The discovery of 312 markers linked to drought and heat response through association mapping analysis, utilizing both whole genome scanning and a candidate gene-based approach, has been documented in chickpea (Thudi et al., 2014). The presence of an extensive array of DNA markers has significantly facilitated successful genetic mapping and QTL analysis, not only in chickpea but also in numerous other legumes. Genetic maps play a crucial role in unravelling intricate traits, especially those related to yield and its contributing factors (Barmukh et al., 2021). Modern breeding strategies proficiently leverage genomic resources to map markers linked to specific traits. Establishing genetic maps is a fundamental phase in the identification of markers associated with particular traits through linkage mapping. Over the recent years, several molecular markers and genetic maps have been formulated (Solanki et al., 2022). These methodologies have successfully linked genes with phenotypic variations in both qualitative and quantitative traits (Al-Khayri et al., 2019). Because of the constraints associated with the use of morphological markers for genetic map development, various molecular markers, including diversity arrays technology (DArT) and single nucleotide polymorphisms (SNPs), have proven to be successful (Thudi et al., 2011). The emergence of high-throughput genomic resources has been pivotal in enhancing genetics, contributing to improvements in chickpea nutritional quality, yield, and tolerance to a diverse source of biotic and abiotic stresses. The rapid progress in chickpea genomics is evident in the development of numerous molecular markers designed for evaluating genetic variability (Al-Khayri et al., 2019).

Marker-Assisted Selection

Exploring genetically superior accessions stands as an essential strategy for germplasm conservation and as a potential source of breeding material for the future (Ahmad et al., 2014). The utilization of marker-assisted selection (MAS) in crop improvement programs has experienced a surge in recent years. A primary advantage of MAS, compared

to traditional plant breeding, lies in the reduced number of generations and population size required for releasing elite cultivars (Castro et al., 2013; Padaliya et al., 2013). The MAS approach not only accelerates the speed of breeding programs but also facilitates gene pyramiding, enabling the combination of desirable quantitative trait loci (QTLs) from multiple parents to develop elite cultivars. To successfully implement MAS in a breeding program, it is necessary to judiciously select the genotype and subsequently conduct phenotypic selection of candidate genes along with their associated markers (Al-Khayri et al., 2019). Despite the development of numerous molecular marker systems, single nucleotide polymorphisms (SNPs) are preferred markers for breeding applications and genetics (Mir and Varshney, 2012). Several molecular markers capable of generating polymorphisms from genic regions of the genotype have been established. These include SRAP (Sequence-Related Amplified Polymorphism) (Li and Quiros, 2001; Kumar et al., 2014); SCoT (Start Codon Targeted Polymorphism) (Collard and Mackill, 2009; Hajibarat et al., 2015); CDBP (CAAT Box Derived Polymorphism) (Singh et al., 2013); TRAP (Target Region Amplification Polymorphism) (Hu and Vick, 2003); and CoRAP (Conserved Region Amplification Polymorphism) (Wang et al., 2008).

Marker-Assisted Recurrent Selection (MARS)

MARS offers a rapid means of advancing generations, incorporating individual genotypic selection, and intercrossing within a single selection cycle. This innovative molecular breeding approach differs significantly from traditional QTL or MAS studies, as it initiates a new mapping study for each breeding population, increases the frequency of desirable alleles in the populations (Al-Khayri et al., 2019). The MARS process commences with a diverse base population and harnesses superior recombinants in each cycle to yield a broadly enhanced population, inbred line, or hybrid (Cholin et al., 2023).

Speed breeding

Typically, breeding programs employ a cycle of selection, recombination, and further selection to cultivate new varieties. This process involves about 5-6 generations before reaching genetic homozygosity, at which point

the varieties are tested for their stability and performance (Roorkiwal et al., 2020). Recent investigations have indicated the potential to achieve 4-6 generations annually in chickpea through speed breeding conditions, incorporating extended photoperiods and controlled temperature regimes (Watson et al., 2018).

Doubled-Haploid Production

The utilization of Doubled Haploid (DH) technology is a highly efficient approach in plant breeding, offering substantial time and cost savings. This method is particularly valuable for swiftly generating pure, entirely homozygous parental lines, a critical requirement for the large-scale production of hybrid seed (Grewal et al., 2009; Seguí-Simarro et al., 2021). The primary advantages of doubled-haploid production include the augmentation of cultivar improvement, increased homozygosity, and better alignment with market demands. However, the success of anther embryogenesis and the subsequent regeneration of complete haploid plants within the *Fabaceae* family is limited to select few species, such as pigeon pea and alfalfa (Croser et al., 2006). The challenging nature of legumes impedes swift progress in haploid plant production. Modern genomic technologies possess the capability to accelerate the procedures involved in trait mapping, marker formulation, gene exploration, and molecular breeding. Furthermore, they play a role in enhancing the rate of productivity gains in chickpea. The merging of genome-wide sequence data with accurate variations in phenotype enables the identification of accessions containing low-frequency variants, potentially responsible for vital traits like yield components, resistance to diseases or tolerance to abiotic stress (Roorkiwal et al., 2020).

The diversity of chickpea by screening of the vast volume of accessions available in gene banks

In recent years, there have been several efforts to integrate all gene banks into a global system for conserving plant genetic resources (Piergiovanni, 2022). Indian landraces are the primary component of the collection. Plant genetic resources (PGRs) present in the gene bank provide the starting point to understanding genetic diversity that can be

used in modern breeding to create cultivars that are highly productive and tolerant to climate changes (Varshney et al., 2013). More than 30 gene banks across the world conserve about 97,400 accessions of chickpea germplasm. Most of these accessions originated in India, Iran, Syria, and Turkey (Chandora et al., 2020). The most extensive collection of chickpeas in the world is held by ICRISAT, which comprises 20,764 accessions, ranging from 20,456 cultivars to 308 wild *Cicer* species collected from 60 countries. At that time, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) held 41.2% of chickpea accessions (Piergiovanni, 2022). The ICARDA gene bank has maintained 15,734 accessions, which include 540 accessions of wild *Cicer* species, within its worldwide germplasm repository from 61 countries. At NBPGR in New Delhi, the Indian National Gene bank maintains 14,704 chickpea accessions. Other gene banks conserve 8655 and 8038 accessions (Chandora et al., 2020). ICRISAT and ICARDA scientists had the advantage of selecting improved varieties based on the availability of large collections during the planning and development of breeding programs. In recent decades, scientists working in both gene banks have released several improved varieties (Piergiovanni, 2022).

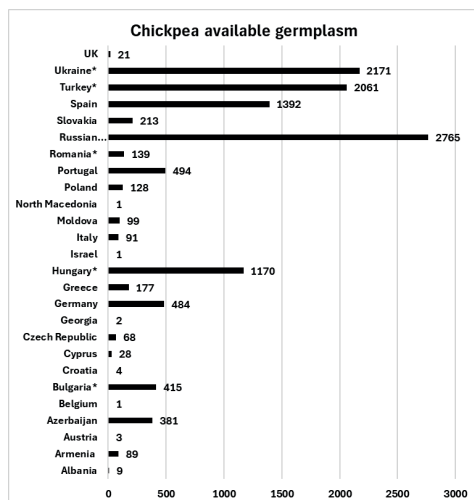


Figure 1. Total number of chickpea accessions from European Search Catalogue for Plant Genetic Resources (EURISCO) <https://eurisco.ipk-gatersleben.de/>, *different varieties

A total of 12,407 chickpea accessions were identified only from the European Search Catalogue for Plant Genetic Resources (EURISCO) (Figure 1). To use these resources efficiently in plant breeding programs, it is necessary to be aware of the extent and distribution of genetic diversity. The need to conserve and manage genetic resources for future breeding attempts is highlighted by shifts in genetic variation because of domestication, crop expansion, and breeding (Mousavi-Derazmahalleh et al., 2019).

Germplasm conservation

The sustainable and effective conservation of crop genetic resources relies on the proactive management of preserved germplasm. Achieving successful germplasm conservation necessitates a comprehensive approach that incorporates both in situ on-farm and ex situ conservation strategies in a well-balanced manner (Rajpurohit and Jhang, 2015).

The aim of ex situ conservation is to maintain the genetic diversity and integrity of species collected outside their natural habitats to prevent their genetic erosion or degeneration. The genetic diversity maintained all over the world in different centres and infrastructures plays a crucial role in breeding. A recent survey on wild and cultivated (landraces, old varieties) accessions of *Cicer arietinum* in Europe versus globally is presented in Figure 2.

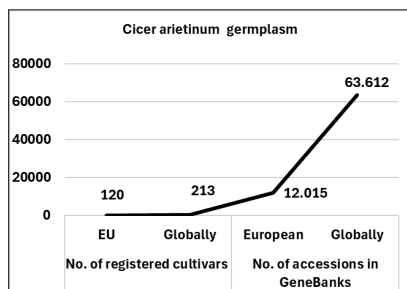


Figure 2 Registered cultivars and conserved accessions of *Cicer arietinum* in EU vs globally according to (Roorkiwal et al., 2020)

Several ongoing breeding programs are focused on the development of phenotypic and genotypic knowledge. It was observed that the selection of varieties during breeding progress, imprinted a decrease of genetic diversity explored in agriculture. In case of the chickpea insufficient diversity for specific emerging

traits was signalled. To reduce this negative effect a continuing infusion of genetic diversity coming from old varieties, landraces, ecotypes, or wild populations is needed (Pratap et al., 2021). The genetic diversity of wild *Cicer* species, including *C. microphyllum*, has been collected by the National Bureau of Plant Genetic Resources (NBPGR) from various locations. The wild varieties of chickpea, such as *C. judaicum*, *C. pinnatifidum*, and *C. bijugum*, have been found to possess different genes that protect against ascochyta blight, botrytis gray mold, and fusarium wilt (Chandora et al., 2020). The active management of conserved germplasm is necessary for sustainable and effective conservation of crop genetic resources. Germplasm conservation requires a holistic approach adopting both conservation strategies. *In situ* conservation is the process of conserving species, landraces, or populations in their natural habitat. Evolutionary processes keep making this system dynamic. Traditional seed conservation practices in traditional farming systems are commonly employed to conserve cultivated species. To conserve as much as is possible of chickpea diversity, it is important to combine in situ and ex situ conservation strategies since ex situ collections cannot contain the entire gene pool (Piergiovanni, 2022).

The role of inclusion of wild genetic material in current breeding programs

The genetic diversity within the cultivated chickpea gene pool is limited, primarily attributed to factors such as domestication bottleneck (Spillane and Gepts, 2001), genetic drift, migration, and the underutilization of genetic resources in chickpea breeding (Kumar et al., 2004). The diversity of wild *Cicer* species holds a wealth of useful alleles that, when identified, can aid in breaking yield barriers, improving resistance to major stresses, and contributing to yield stability. By introducing related wild *Cicer* species to cultivated chickpea, useful genes will be included in cultivated varieties, resulting in the inclusion of these wild species in common gene pools (Singh et al., 2014). As an alternative genetic source for crop improvement, breeders are examining the wild relatives of chickpea.

The *Cicer* genus' evolutionary relationships have been better understood through research in recent years, and novel technologies have been developed to help transfer genes from the wild to the cultivated species (Croser et al., 2003). The *Cicer* genus has 43 species, so hybridizing cultivated species with unimproved wild relatives can result in an increase in genetic diversity (Croser et al., 2003). The distribution pattern of wild relatives of crops offers crucial insights, revealing potential areas of domestication. In the case of chickpeas, wild relatives are predominantly located in Turkey, Syria, Lebanon, India, Afghanistan, Ethiopia, Israel, Jordan and Pakistan (Chandora et al., 2020). Due to the harsh climate conditions in the regions where many of these species are endemic, perennial species are likely to be particularly beneficial for abiotic stress tolerance breeding. The relationship between wild *Cicer* species and chickpea breeding programs must be well understood to be successful. The analysis of genomic relationships and the construction of phylogenies among plant species has traditionally been conducted using phenotypic traits, hybridization, analysis of chromosome pairing in hybrids, and the study of chromosome structure (Croser et al., 2003). The development of chickpea varieties began with the use of native or introduced landraces in the early stages. Hybridization has been used to develop most recent varieties. The wild *Cicer* species are particularly valuable gene pools due to their ability to resist both biotic and abiotic stresses (Table 1). Cross-border barriers have kept these from being fully utilized, but there have been some successful gene introgression examples into cultivated species from two closely related species, *C. reticulatum* and *C. echinospermum* (Gaur et al., 2012). Fungi and nematodes are the main causes of biotic stress that threaten the legume crop. Chickpea faces significant problems from root-knot, cyst, and root-lesion nematodes, resulting in a combined annual yield loss of around 14% (Ali et al., 2022). The genetic diversity within cultivated chickpeas limits resistance to nematode species, yet several wild chickpea species exhibit notable levels of resistance to these nematodes.

Table 1. Abiotic and biotic stress response of the annual wild relatives of domesticated chickpea

Sp.	Abiotic stress response	Biotic stress response	References
<i>C. reticulatum</i>	Tolerant to low temperature, drought and heat	Tolerant to Cyst nematode	Nene and Haware, 1980; Toker et al., 2007; Talip et al., 2018;
<i>C. echinospermum</i>	Tolerant to low temperature	Tolerant to Fusarium wilt, Ascochyta blight, Phytophthora root rot, Bruchids	Singh et al., 1981, 1998; Toker, 2005; Berger et al., 2012; Talip et al., 2018;
<i>C. bijugum</i>	Tolerant to low temperature	Tolerant to Bruchids, Ascochyta blight, Cyst nematode, Fusarium wilt	Singh and Weigand, 1994;
<i>C. cuneatum</i>	Sensitive to low temperature	Tolerant to Leaf miner, Fusarium wilt	Singh and Weigand, 1994; Singh et al., 2008;
<i>C. chorassanicum</i>	Sensitive to low temperature	Tolerant to Leaf miner	Singh and Weigand, 1994; Singh et al., 2008;
<i>C. pinnatifidum</i>	Tolerant to drought and low temperature	Tolerant to Gray mold, Cyst nematode, Fusarium wilt, Ascochyta blight	Singh et al., 1982; Toker, 2005; Toker et al., 2007;
<i>C. judaicum</i>	Sensitive to low temperature	Tolerant to Fusarium wilt, Ascochyta blight, Leaf miner, Gray mold, Bruchids	Singh et al., 1998; Toker, 2005;
<i>C. yamashitae</i>	Sensitive to low temperature		Toker, 2005;

Barriers to interspecific hybridization impede the utilization of specific wild species as sources of nematode resistance.

However, certain species like *C. reticulatum* and *C. echinospermum* have proven to be significant sources of nematode resistance genes (Zwart et al., 2019; Jimenez-Lopez et al., 2020).

The only known resistance for bruchid (*Callosobruchus chinensis*) and cyst nematodes is found in wild *Cicer* species, and they exhibit greater resistance than cultivated plants against fusarium wilt, botrytis grey mold, leaf miner, and cold. The fact that a few *Cicer* species accessions can resist three or more stresses is even more significant, as no line of *C. arietinum* has been proven to be resistant to more than one stress in ICARDA evaluations. The following accession, *C. reticulatum*, *C. echinospermum*, *C. bijugum*, *C. judicarium*, and *C. pinnatifidum*, are particularly promising and can survive 4 or 5 different stresses. It is evident that these accessions would serve as excellent candidates for interspecific hybridization actions. Among them, *C. bijugum*, *C. pinnatifidum*, and *C. judaicum* stand out as the three species with accessions showcasing the highest resistance to various stresses in terms of performance (Croser et al., 2003). The investigation conducted by Zhou et al. (2019) explored the differential expression of 10 Resistance Gene Analogs (RGAs), which are significant in the recognition of plant pathogens and the signalling of inducible defences, are expressed differently in cultivated chickpea varieties that are either resistant or susceptible to the foliar disease *Ascochyta* blight caused by the fungus *Ascochyta rabiei* (syn. *Phoma rabiei*). ICC3996 was the genotype with the highest level of resistance to inoculation, spore germination, and penetration into the plant's epidermal tissues, and four RGAs were consistently upregulated, leading to significant differential expression of four RGAs. Future functional validation and selective resistance breeding for introgression into elite cultivars can be achieved through these clear targets (Jimenez-Lopez et al., 2020). Cultivars that are resistant to various diseases, including *Ascochyta* blight and *Fusarium* wilt, have been developed and introduced in numerous countries. The ICRISAT Centre has developed lines that are resistant to *Helicoverpa*, but none of them have been released as cultivars because they are

susceptible to *Fusarium* wilt. Resistance to *Ascochyta* blight is also present in cold-tolerant lines developed at ICARDA. Many countries have seen their release. Australia has developed lines that can resist *Phytophthora* root (Bithell et al., 2018). Until now, there has been no sole focus on breeding drought-resistance. Different development stages are present for breeding efforts to resist nematodes, viruses, root rot diseases, leaf miner, and heat (Singh et al., 1993). Indian varieties are well-suited for studying molecular mechanisms of drought tolerance due to their special adaptation to drought and high temperature stress. The drought-resistant mechanism of the ICCV 2 genotype of kabuli chickpea, which ICRISAT has released, has proven to be a success (Rasool et al., 2015). Heat stress accelerates the initiation of flowering, pod development, and maturation in a progressive manner. Additionally, it induces leaf senescence and influences various yield-related factors such as harvest index (HI) (Devasirvatham et al., 2015). Chickpea employs strategies like escape, avoidance, and tolerance to cope with heat stress. Early maturing genotypes can evade late-season heat stress, whereas late-maturing ones are vulnerable during crucial stages like flowering and podding, potentially leading to reduced yields. There is a negative correlation between days to flowering and yield, with pod number per plant and HI showing the strongest associations with grain yield under heat stress (Kaushal et al., 2013). ICARDA is working on transferring genes for resistance to cold and cyst nematode from *C. echinospermum* and *C. reticulatum*, but the most important resistance sources are the wild species *C. bijugum*, *C. judaicum*, and *C. pinnatifidum*. The cultigen has not been able to be crossed with these wild species. As a result, there has been no effort to transfer genes from these species (Singh et al., 1993). Singh et al. (1993) conducted a study at The Punjab Agricultural University in Ludhiana, India, aiming to hybridize four cultivars (three desi and one kabuli) of cultivated species with five annuals wild *Cicer* species (*C. judaicum*, *C. judaicum*, *C. pinnatifidum*, and *C. reticulatum*) using an interspecific approach. In the crosses, wild species were used as male parents while cultivars were used as female parents. The

female genotype and the specific combination with the wild species played a crucial role in crossing success. There was no success with the kabuli cultivar as a female parent. *C. bijugum* had the lowest overall success while *C. reticulatum* had the highest.

The wild chickpea and the cultivated variety have distinct differences in their plant growth habit, altered phenology, seed coat texture, and reduced seed dormancy (Hammer, 1984).

The types of cultivated chickpea are classified based on their seed shape, size, and colour variation.

- Desi type (microsperma), among other characteristics small seed types, bushy growth habits, pink flowers, anthocyanin pigment, angular seed shapes with dark colour, and rough seed coats, of which is predominantly grown in semiarid tropics, are characteristics of this type of plant. The Desi type is regarded as an earliest version of the cultivated chickpea that has been domesticated (Ladizinsky and Adler, 1976). Desi chickpea production is predominately from Desi type, accounting for about 85% of world production (Figure 3).



Figure 3. Plant pigmentation, seeds and flowers of chickpea Desi type – experimental fields of VRDS Bacău



Figure 4. No pigmentation plant, seeds and flowers of chickpea Kabuli type – experimental fields of VRDS Bacău

- Kabuli type (macrosperma) is a species that is mostly confined to temperate regions of Mediterranean countries. It has white flowers with an owl-shaped head seeds, and it lacks anthocyanin pigment. The seed coat

is smooth and white or beige, and it grows upright with large seeds that have low fibre content (Figure 4). Kabuli is a derived version of desi because the seeds of the desi type are more resembling those of wild *C. reticulatum* than those of the Kabuli type. Within cultivated chickpea, both types are grouped into two interconnected clusters that represent two different gene pools (Chandora et al., 2020). Kabuli chickpea production accounts for about 15% of the world's production (Chandora et al., 2020).

CONCLUSIONS

The main interest of the breeding programmes is to develop new germplasm able to perform under the pressure of the main biotic stresses that affect the productivity. *Fusarium* wilt and *Ascochyta* blight are frequently reported and having a negative influence on yield. In addition, focus on pod bored, botrytis grey mould is increasing due their significant pressure on chickpea yields. In terms of abiotic stresses, drought, and cold tolerance is observed in many regions. Despite the efforts to develop new yielding cultivars by conventional breeding programmes, the application of genomics technologies is imperative needed having the benefit to speed up the new cultivar development. Marker-assisted selection is facilitating the selection of favourable alleles. Integration of genetic maps and whole-genome sequence information represent a sustainable strategy for the current and future breeding programs that ensures the development of new resilient materials, able to perform in different climatic contexts.

Exploring the diversity of chickpea based on a holistic approach on *in situ*, *ex situ* and *on farm conservation* has a significant potential to enhance the role of wild genetic material inclusion to ensuring tolerance/resistance to different abiotic and biotic stressors and to improve yields.

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